

MESOSCALE STRUCTURE OF ZOOPLANKTON IN THE CALIFORNIA CURRENT

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Award # N00014-1-96-0302 (UNH); N00014-1-96-0421 (RSMAS);
N00014-1-96-0268 (WHOI)

LONG-TERM GOALS

The long range goal of this collaboration is to develop a better understanding of zooplankton population dynamics, especially at the boundaries of distributions, through the use of specifically-designed field efforts utilizing bio-acoustics, biochemical and molecular analyses, and process models.

OBJECTIVES

We are examining how populations of euphausiids maintain high concentrations in coastal regions of the California Current despite the highly advective field. We are testing the hypothesis that mesoscale eddies and coastal counter currents create retention cells, where growth and reproduction are rapid and mortality is reduced. The immediate objective is to produce a data set on the biomass, population genetics, and physiological condition of the targeted euphausiids across the boundary between the eutrophic inshore of the California current and the oligotrophic central gyre waters. This work is supported by ONR Biological Oceanography.

Report Documentation Page				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 30 SEP 1997		2. REPORT TYPE		3. DATES COVERED 00-00-1997 to 00-00-1997	
4. TITLE AND SUBTITLE Mesoscale Structure of Zooplankton in the California Current				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of New Hampshire, Ocean Process Analysis Laboratory, Durham, NH, 03824				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 4	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

APPROACH

We are using a suite of techniques - including molecular genetics, enzyme biochemistry, and bioacoustics - to examine the mechanisms that define the boundaries of zooplankton species' distributions in the California Current. We are analyzing the physiological and population genetic characteristics of euphausiids of the target species in offshore and nearshore regions of the California Current. The physiological characteristics are enzyme activities and the genetic characteristics are molecular variants of temperature-sensitive enzymes of euphausiids along CalCOFI line 83.3. The field work is accompanied by the formulation of a set of models of these animals that explicitly considers selection on nuclear genetic traits and the effect of this selection on population structure.

WORK COMPLETED

Field collections: An additional set of vertically-stratified MOCNESS samples was obtained during August 1997 from the California Current, as part of the ONR-funded BioAcoustics course on the R/V Revelle (cruise KIWI-1). These samples will be used for comparison with samples from cruise RR-9610 along CalCOFI line 83.3. As previously, samples were split and preserved in formalin or alcohol.

Molecular genetics: Individual *Nematocelis megalops* were identified in the alcohol-preserved samples from the 15 MOCNESS tows taken during RR-9610, removed to individual vials, and prepared for molecular analysis. A 450 base-pair region of the mitochondrial cytochrome oxidase I (COI) gene was sequenced for a total of 110 individuals. The sequence data are being analyzed for evidence of population genetic structure by several tests, including a hierarchical analysis of molecular variation (AMOVA) comparing variation within and between samples and regions (i.e., coastal, transition, and offshore). Sample sizes are sufficient for a valid population genetic analysis (i.e., more than 30 per region).

After some deliberation about technical approaches to identify a trait encoded in the nuclear genome that is likely to be subject to selection, we decided to use differential display, reverse transcriptase PCR (dd-rtPCR) to examine genes that are differentially expressed (i.e., turned on-or-off) in different populations and environmental conditions. We have extracted usable mRNA from individually-frozen *N. difficilis* collected during RR-9610, and are currently adapting established dd-rtPCR protocols to euphausiids. The dd-rt PCR assays require only a small amount of tissue; the biochemical and genetic analyses were thus done using the same individuals (see below).

Biochemical analysis: Enzyme kinetic assays were completed for 100 individuals of *N. difficilis* collected during RR-9610. The enzymes assayed were: citrate synthase (CS), lactate dehydrogenase (LDH), phosphoglucose isomerase (PGI), and hexokinase (HK). In addition, the same analyses were done for 300 individuals of several other euphausiid species. Protein analyses were conducted on all samples; all enzyme values were scaled to protein. These data will be analyzed to determine the physiological condition and growth of individual euphausiids across the sampled domain.

High-frequency acoustics: In order to groundtruth the bioacoustics data, the taxonomic composition and size frequency distribution of zooplankton were determined for MOCNESS samples from RR-

9610 by silhouette analysis. Silhouette analysis was completed for 4 day and 4 night samples collected in the upper 100 m. The analysis of these data is continuing. The results will provide the biomass context for the single-species molecular and biochemical analyses.

In August 1997 aboard the R/V Revelle (cruise KIWI-1), an HTI two-frequency (120 and 200 kHz) echosounder, with transducers towed in a down-looking mode at 3-5 m depth, was used to acquire volume backscattering data along the same trackline that was sampled in October, 1996. MOCNESS tows were taken at the start and the end of the acoustic section. The MOCNESS samples were split equally, with half preserved in formalin for silhouette analysis to groundtruth the bioacoustics data and half preserved in alcohol for molecular genetic analysis.

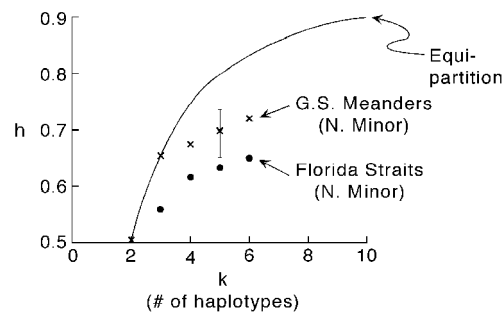
Modeling: A schematic of a model to treat the impact of enzyme expression on a planktonic organism is finished. The process of simplifying the model and testing it for compatibility with the observations will begin in the next funding cycle. Also, a set of physical models covering upwelling systems was developed based on the Miami Community Ocean Model (MICOM). Three types of plankton models are currently up and running in these simulations. The first two are a simple NPZD model and a multiple foodchain model that is also constructed with nutrients as a conservative currency. These models are used as a background age (stage) and metabolically structured model that runs on particles. This Lagrangian model also has the capability of simulating population genetics as demonstrated in the paper submitted to JMR.

RESULTS

Oceanographic setting: The hydrographic analysis from the domain sampled during RR-9610 indicated the presence of a coastal eddy transected by CalCOFI line 83.3, which was the premise of our proposal. Based on surface hydrography and circulation, our transect of 15 MOCNESS tows sampled from offshore, nutrient-poor waters; a transition region of intermediate properties; and coastal, nutrient-rich waters (see RR-9610 cruise report, T. Hayward, ed.).

Molecular genetics: Preliminary examination of the DNA sequence data for a 350 base-pair region of the mitochondrial COI gene indicated that there is significant sequence variation within *N. difficilis* collected along CalCOFI line 83.3 in October, 1996. The sequence data also clearly separated *N. difficilis* from its Atlantic sibling species, *N. megalops*. Patterns of geographic variation of molecular population genetic

Figure 1. The distribution of haplotype diversity versus the number of haplotypes included by rank order of abundance from observations in the Florida Straits and Gulf Stream meander region. Also shown is the diversity one would get if all haplotypes are of equally abundant (equipartition) and the simulation results (vertical bar at $k = 5$). The comparison is fairly good, although it suggests that population carrying capacity for this copepod in the southern gyre is less than used in the model. More observations are needed in the Caribbean to test this suggestion.



variation have not yet been determined. In addition, we have established that individually-frozen *N. difficilis* yield high-quality mRNA for analysis of differential gene expression. Thus, we are confident that we will be able to locate a nuclear genetic trait that will indicate the impact(s) of natural selection on the populations.

Biochemical analysis: Frozen samples of *N. difficilis* and other euphausiids have yielded high-quality data on the physiological condition of individual euphausiids. Activities of all enzymes were measurable and high. We will thus be able to complete our proposed synthesis of biochemical analysis of condition and growth and molecular genetic analysis of dispersal and selection.

High-frequency acoustics: A portion of the RR-9610 transect along CalCOFI line 83.3 was repeated during the October 1997 cruise of the R/V Revelle (KIWI-1). The same high-frequency bioacoustic instrumentation was used for both visualizations of the transect; MOCNESS samples were collected for analysis and groundtruthing during both cruises. The repetition of the transect will allow some assessment of temporal variation in the patterns observed.

Modeling: A model of zooplankton genetics in an ocean gyre was completed and a manuscript was submitted to the Journal of Marine Research. The analysis revealed significant spatial pattern in the population genetic character of zooplankton as they are advected through varying environmental carrying capacities. The model results were consistent with the observations in the Gulf Stream system (Fig. 1), based on a published study of the copepod, *Nannocalanus minor* (Bucklin et al., 1996, J. Mar. Res. 54:285).

IMPACT

Our fundamental approach, which combines experimental data and modeling, should provide new insights into interpretation of population dynamics in the ocean. In addition, the integrated analysis of biochemical, molecular, and high-frequency acoustic data will provide new information for biological oceanographers who seek to understand complex processes and will also provide new indices for rapid assessment of the planktonic assemblage. The impact of this work will be to improve biological models of ocean assessment and prediction.

TRANSITIONS

The model codes will be made widely available after they have been further tested and reviewed for publication.

RELATED PROJECTS

The October 1997, cruise of the R/V Revelle (KIWI-1) was both an educational and a research venture, with support from ONR and the University of California at Santa Cruz. This work has also been supported on the intellectual side through the ONR URIP on marine population dynamics.